PATENT

-- 37. (New) The method of claim 36, wherein the negatively selectable marker encodes thymidine kinase. --

-- 38. (New) The method of claim 35, wherein the plasmid is delivered to the cell by electroporation, microinjection or transformation. --

REMARKS

With entry of this amendment, claims 14 and 29-38 are pending in the instant application. By this amendment, claim 14 has been amended for clarity in accordance with the Examiner's suggestions and to correct certain obvious typographical and clerical errors. In particular, claim 14 is amended to further clarify that the animal is a non-human animal. Support for this amendment is found throughout the instant specification, such as, for example, at page 4, lines 14-17 and at page 15, lines 7-14. Claim 14 is also amended to recite that the gene encoding $p27^{Kip1}$ is altered in a somatic cell of the animal to cause a functional deficiency of cyclin-dependent kinase inhibitor function of $p27^{Kip1}$. Support for this amendment is found throughout the instant specification, such as, for example, at page 4, lines 6-12; page 13, lines 2-7; page 13, lines 18-35; page 14, lines 15-30; and page 15, lines 7-14.

Entry of new claims 29-38 is respectfully requested; new claims 29-38 depend, directly or indirectly, from independent claim 14.

New claim 29 recites that the somatic cell is a thymocyte or bone marrow cell. Support for this new claim is found throughout the instant specification, such as, for example, at page 18, lines 15-22.

New claim 30 recites that the non-human animal is a mouse, rat, pig, sheep, frog, cow or bull. Support for this new claim is found throughout the instant specification, such as, for example, at page 15, lines 7-14.

New claim 31 recites that the altered gene encoding $p27^{Kip1}$ is disrupted by insertion of a positively selectable marker into the gene, or a mutation or deletion in the gene. New claim 32 recites that the altered gene encoding $p27^{Kip1}$ is altered by insertion of a positively selectable marker into the gene. Support for these new claims is found throughout

the instant specification, such as, for example, at page 15, lines 25-29 and at page 16, lines 8-22.

New claim 33 recites that the positively selectable marker encodes neomycin resistance, thymidine kinase, adenine phosphoribosyl transferase, hypoxanthine-guanine phosphoribosyl transferase or dihydrofolate reductase. New claim 34 recites that the positively selectable marker encodes neomycin resistance. Support for these new claims is found throughout the instant specification, such as, for example, at page 13, lines 22-35.

New claim 35 recites that the method further comprises introducing a plasmid into the cell, the plasmid comprising the gene encoding $p27^{Kip1}$ altered by insertion of a positively selectable marker. Support for this new claim is found throughout the instant specification, such as, for example, at page 4, lines 27-37; at page 13, lines 22-35; and at page 16, lines 8-22.

New claim 36 recites that the plasmid of claim 35 further comprises a negatively selectable marker. New claim 37 recites that the negatively selectable marker encodes thymidine kinase. Support for these new claims is found throughout the instant specification, such as, for example, at page 4, lines 27-37, and at page 16, lines 8-28.

New claim 38 recites that the plasmid is delivered by electroporation, microinjection or transformation. Support for this new claim is found throughout the instant specification, such as, for example, at page 15, line 36 to page 16, line 22.

All of the amendments and new claims presented herein are fully supported by the specification, and no new matter has been added to the application. Entry of these amendments and new claims is respectfully requested.

Applicants acknowledge that the Examiner has withdrawn the objection under 35 USC § 119 and that the application properly claims priority to International Application No. PCT/US97/05921, filed April 10, 1997, and the benefit of U.S. Provisional Application No. 60/015,097, filed April 10, 1996.

Amendment to the Specification

The Examiner acknowledged entry of the amendments to the instant specification that were requested in the response to the last office action (mailed April 4, 2000), except for a requested correction at page 16, line 11 (changing "exist" to -- exists--). Applicants inadvertently requested the same correction to page 15, line 11 and to page 16, line 11. The requested amendment to claim 16, line 11, was made in error. Applicants apologize for any confusion.

Abstract

The Examiner again requested submission of the abstract, although Applicants submitted the abstract with the filing of the current application. To proceed with more compact prosecution of this case, Applicants are submitting a copy of the abstract, as filed with the application.

Rejection Under 35 U.S.C. § 112

Claim 14 stands rejected under 35 U.S.C. § 112, first paragraph, the Examiner believing that the specification, while being enabling for inhibition of $p27^{Kip1}$ in a mouse model, allegedly does not reasonably provide enablement for increasing the proliferation of thymocytes (thymic T-cells) in any animal, for the reasons of record as set forth in the official action mailed October 4, 1999.

The first paragraph of 35 U.S.C. §112 requires that the instant specification contain a written description of the invention, and of the manner and process of making and using the invention, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention. Some experimentation may be required, but any experimentation cannot be "undue." In re Wands, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Further, Applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. In re Angstadt, 190 USPQ 214, 218 (CCPA, 1976). What is required is that there is sufficient disclosure, either through illustrative examples or terminology, to teach those of skill how to make and how to use the invention as broadly as it is claimed.

Applicants respectfully traverse the rejection of claim 14 under 35 U.S.C. § 112, first paragraph. The Examiner first reasons that the instant specification, while enabling for inhibition of p27^{Kip1} in a mouse model, does not reasonably provide enablement for increasing the proliferation of thymocytes in any animal. Applicants respectfully remind the Examiner that Applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. The instant application discloses methods for inhibiting p27^{Kip1} in both somatic and germline cells. See, for example, page 13, line 1 through page 17, line 10, and throughout the detailed description of the invention, wherein methods are generally provided for making non-human, transgenic animals, not limited exclusively to mice, and wherein other methods for inhibiting p27^{Kip1} are provided. p27^{Kip1} can be inhibited by gene disruption and by the administration of therapeutic agents, such as by administration of an antibody or a portion of an antibody directed against p27 protein. While specific examples provided in the specification are directed to the production of transgenic mice, the Examiner will appreciate that such teachings are sufficient to teach a person of ordinary skill in the art that inhibition of p27 interaction with the cyclin/CDK complex increases the number of thymic T-cells (thymocytes) which traverse the cell cycle. Inhibition of the interaction between p27 and the cyclin/CDK complex can be accomplished in a number of ways including reducing the amount of p27 by making a transgenic animal, i.e., transgenic mice and other transgenic animals, and by providing agents which prevent the formation of the p27/CDK complex.

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The Examiner further believes that the specification, while teaching by way of example decreasing expression of p27^{Kip1} by creation of a p27 mouse knockout, does not provide examples of methods of administering a therapeutic agent which functions in a whole organism to decrease levels of p27^{Kip1}. Applicants respectfully traverse this basis for rejecting claim 14. Applicants' claims simply require treating the animal to increase the proliferation of thymocytes (thymic T-cells) in the animal. The recited increase in thymocyte proliferation can be achieved by causing a functional deficiency of the cyclin-dependent kinase inhibitor function of p27^{Kip1} in thymocytes, or thymocytes precursors, such as bone marrow cells. (See Specification at page 16, line 30 to page 17, line 25.) Further, as disclosed in the instant specification, the cyclin-dependent kinase inhibitor function of p27^{Kip1}

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can be decreased by administering an agent locally or to the whole organism. The claims are not limited to administration of the agent to the whole organism, as reasoned by the Examiner.

The Examiner further disagrees with Applicants' discussion of the Crystal reference. Applicants respectfully note that the Crystal reference, cited by the Examiner as teaching the unpredictability of gene therapy, does indeed state that "most studies have shown that genes can be transferred to humans whether the strategy is *ex vivo* or *in vivo*, and that all types of vectors function as intended." (See Crystal at page 405.) Crystal concludes that "[t]aken together the evidence is overwhelming, with successful gene transfer having been demonstrated in 28 *ex vivo* and 10 *in vivo* studies." (See id.) Crystal then summarizes *ex vivo* and *in vivo* studies showing successful gene transfer. (See id. at pages 405-07.) Crystal further summarizes relevant biological responses to *ex vivo* and *in vivo* studies. (See id. at pages 405-07.) While acknowledging that "[n]o human disease has been cured by human gene transfer," Crystal teaches that "several studies have demonstrated that therapeutic genes transferred to humans by means of retrovirus, adenovirus, and plasmid-liposome vectors can evoke biological responses that are relevant to the gene product and the specific disease state of the recipient." Crystal then discusses several examples of *ex vivo* or *in vivo* treatments, including treatment of SCID ADA deficiency, familial hypercholesterolemia, cystic fibrosis and tumor vaccine studies." Hence, Crystal not only teaches that gene therapy is feasible, but that it does indeed work.

The Examiner cites as evidence of unpredictability a statement by Crystal at page 409, in which Crystal states that "[a]ll of the human gene transfer studies have been plagued by inconsistent results, the bases of which are unclear." These studies relate to therapeutic efficacy. In particular, the Examiner points to the ADA deficiency example, where Crystal reports that the proportion of autologous T-cells modified *ex vivo* with the normal ADA cDNA resulted in proportions of ADA^+ circulating T-cells varying from 0.1 to 60%. Applicants respectfully note that even at 0.1%, there would be a significant increase in the total number of ADA^+ circulating T-cells. Similarly, for the cystic fibrosis trials, an increase of at most 5% of the target T-cells, the respiratory epithelium, was reported. Applicants again note that even 5% of the respiratory epithelium is a significant number of

treated cells. Further, such treatments were based on whole organism treatments. The Examiner will also appreciate that localized treatments would be expected to produce higher levels of biological response.

The Examiner also reasons that "[a] great deal of trial and error experimentation is required to achieve a small amount of success, such as the 'expression observed in at most 5% of the target T-cells ....'" Applicants respectfully note that there is no support in Crystal for this statement. Assuming that some "trial and error experimentation" were required, Crystal does not make any statements supporting the Examiner's assertion that a "great deal" would be required.

Applicants also note that the Examiner appears to incorrectly equate therapeutic efficacy with a useful increase in the proliferation of thymocytes (thymic T-cells). In so equating therapeutic efficacy with a useful increase in the proliferation of thymocytes, the Examiner improperly reads additional limitations into the claims. Applicants' claims only require a useful increase proliferation of thymocytes. Therapeutic efficacy is an FDA requirement, not a requirement for patentability. Applicants also believe that the Examiner misinterprets a suggestion in Crystal or Anderson of low efficiency as suggesting unpredictability. Low efficiency simply denotes that a larger number of subjects must be treated per successful increase event, and not that success is unlikely (i.e., unpredictable). Hence, a low efficiency gene therapy protocol can be predictable, while high efficiency protocols can be unpredictable. In contrast, low efficiency gene therapy protocols are regarded as having low therapeutic efficacy, while high efficiency protocols are regarded as having high therapeutic efficacy. In other words, while a therapeutic efficacy of 5% or 0.1% may be considered too low for FDA approval, an increase of 60%, 5% or even 0.1% in thymocyte proliferation is useful to the subject. Applicants believe that the methods disclosed in the instant application, which range from gene alteration to the administration of various agents, are predictable, although resulting in a range of efficiencies according to the particular method being used. Thus, Applicants have properly enabled an increase in thymocyte proliferation, even assuming arguendo that Applicants have not enabled therapeutic efficacy of thymocyte proliferation.

The Examiner further reasons that neither the art nor the specification provides sufficient guidance to one of skill in the art to design and administer gene therapeutic agents to the claimed target gene for the claimed effects, except via gene knockout. The instant application discloses methods for inhibiting p27^{Kip1} in both somatic and germline cells. See, for example, page 13, line 1 through page 17, line 10, and throughout the detailed description of the invention, wherein methods are generally provided for the administration of therapeutic agents, such as by administration of an antibody or a portion of an antibody directed against p27 protein. While specific examples provided in the specification are directed to the production of transgenic mice, the Examiner will appreciate that such teachings are sufficient to teach a person of ordinary skill in the art that inhibition of p27 interaction with the cyclin/CDK complex increases the number of thymic T-cells (thymocytes) which traverse the cell cycle.

The Examiner also objects that the gene disruption "technique has limitations in any other whole organism in which stem cells are not available for creation of an organism having all of the target gene knocked-out. Applicants respectfully remind the Examiner that gene disruption can also be performed in somatic cells, and that such cells can be implanted in an organism. In particular, thymocytes are transducible (see, e.g., Akkina et al., Blood 84:1393-98 (1994)), and techniques for introducing gene-disrupting mutations into somatic cells are well known. (See, e.g., Sambrook et al., Molecular Cloning A Laboratory Manual (Cold Spring Harbor Laboratory Press (1989)), Chs. 15-16.) It is also well known that thymocytes can be transplanted into various animals. (See, e.g., Akkina et al., Blood 84:1393-98 (1994).) Thus, contrary to the Examiner's reasoning, Applicants' claims are not limited to the availability of stem cells, but encompass somatic cells as well.

The Examiner also reasons that "the administration of any drug therapeutic agents, whether gene-therapeutic vectors or antisense oligonucleotides, have clear and specific problems in the art and such low levels of reproducibility in technique that one skilled in the art would necessarily practice high levels of trial and error experimentation to make and use a gene therapeutic agent for the claimed functions." In particular, the Examiner reasons that Crystal and Anderson present the overall problems due to expression [sic - inactivation] levels, toxicology and difficulty targeting specific cells types in whole organism.

Applicants respectfully note that toxicology relates to therapeutic efficacy, and should not be read as an additional limitation of the present claims. Applicants have already discussed (supra) that even low inactivation levels can be sufficient to provide a useful increase in the proliferation of thymocytes (thymic T-cells). Finally, Applicants have also explained that the asserted difficulties in targeting specific cells types can be overcome by localized treatment, *ex vivo* therapy and/or treatment of somatic cells.

Finally, the Examiner asserts that the basis for the assertion of unpredictability in the art is not made on the teaching of one reference, but on a broad sweep of the art. In particular, the Examiner reasons that Crystal and Anderson outline the difficulties in the design and administration of gene therapeutic agents to whole organisms. As discussed above and in the response to the previous office action, Applicants respectfully disagree with the Examiner's belief that both Crystal and Anderson teach that gene therapy is so highly unpredictable and limited in successes that the methods are difficult to reproduce and unpredictable. Neither reference teaches that the successes are so highly unsuccessful or unpredictable.

Without acquiescing to the Examiner's rejection, and to proceed with more compact prosecution of this case, Applicants amend claim 14 to recite that the proliferation of thymocytes (thymic T-cells) is increased in a non-human animal by altering the gene encoding $p27^{Kip1}$ in a somatic cell of the animal to cause a functional deficiency of the cyclin-dependent kinase inhibitor function of $p27^{Kip1}$. The gene encoding $p27^{Kip1}$ can be altered by deletion, mutation or insertion of nucleotides into the gene, as described, for example, in the instant specification at page 13, line 18 to page 14, line 36. Methods for altering genes by deletion, mutation or insertion were also well known prior to the priority date of the instant application. (See, e.g., Sambrook et al., Molecular Cloning A Laboratory Manual (Cold Spring Harbor Laboratory Press (1989)), Ch. 15.) Altered genes can be introduced into a somatic cell by microinjection, electroporation or transformation, as well as other techniques known in the art for introducing alterations into gene sequences. Such techniques are described throughout the instant specification, such as, for example, at page 15, line 31 to page 16, line 28. Techniques for introducing altered segments of DNA into mammalian cells were also well known prior to the priority date of the instant application. (See, e.g.,

Sambrook et al., Ch. 16.) In particular, DNA segments can be readily introduced into thymocytes, and transduced thymocytes can be introduced into animals. (See, e.g., Akkina et al., Blood 84:1393-98 (1994).) Thus, Applicants believe that claim 14, as amended, is fully enabled by the instant specification, and complies with 35 U.S.C. § 112, first paragraph.

Applicants also add new claims 29-38 to the instant application. These claims depend, directly or indirectly, from independent claim 14. New claim 29 recites that the somatic cell can be a thymocyte or bone marrow cell. New claim 30 recites that the animal can be a mouse, rat, pig, sheep, frog, cow or bull. New claims 31 and 32 recite that the gene encoding $p27^{Kip1}$ is altered by insertion of a positively selectable marker, mutation of coding sequences of the gene encoding $p27^{Kip1}$, or deletion of coding sequences of the gene encoding $p27^{Kip1}$. New claims 33 and 34 recite that the positively selectable marker encodes neomycin resistance, thymidine kinase, adenine phosphoribosyl transferase, hypoxanthine-guanine phosphoribosyl transferase or dihydrofolate reductase. New claim 35 recites that the method further comprises introducing a plasmid into the cell, the plasmid comprising the gene encoding $p27^{Kip1}$ altered by insertion of a positively selectable marker. New claim 36 recites that the plasmid further comprises a negatively selectable marker adjacent the altered gene encoding $p27^{Kip1}$, whereby the distance between the negatively selectable marker and the altered gene encoding $p27^{Kip1}$ is sufficient to allow homologous recombination. New claim 37 recites that the negatively selectable marker encodes thymidine kinase. New claim 38 recites that the plasmid can be delivered to the somatic cell by electroporation, microinjection or transformation. None of these new claims add new matter to the instant application. Because amended claim 14 is allowable, these claims are also allowable.

In view of the foregoing remarks and the claim amendments, Applicants respectfully request reconsideration and withdrawal of the rejection of claim 14, and the entry and allowance of new claims 29-38.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If the Examiner

believes a telephone conference would aid in the prosecution of this case in any way, please call the undersigned at 206-467-9600.

Respectfully submitted,

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